

I. ON SOUTH AFRICAN PARAMPHISTOMIDAE (Fisch.).

II. SOME TREMATODES IN SOUTH AFRICAN ANURA, AND THE RELATIONSHIPS AND DISTRIBUTION OF THEIR HOSTS.

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(With nine Text-figures.)

I. ON SOUTH AFRICAN PARAMPHISTOMIDAE (Fisch.).

The occurrence of Paramphistomids (=Amphistomes) in sheep and cattle is fairly general in South Africa wherever suitable conditions prevail to ensure its life-history. The conditions required are identical with those required for the life-cycle of the common liver-fluke, viz. swampy vleis where there is a fairly permanent water-supply to ensure both the development of the eggs and the existence of the intermediate host, which in the case of *P. calicophorum* is the ubiquitous *Isidora tropica* Krauss.

These light, flesh-coloured parasites, from 8 to 10 mm. long, occur singly or in groups covering a few square inches in the rumen of sheep and cattle. They attach themselves by the well-developed posterior sucker to the mucous membrane of the rumen between the numerous short villi. The eggs are shed in the stomach, and gradually find their way down the intestine, and are deposited with the fæces. When the fæces with the eggs happen to be dropped in swampy vleis, or in such places where they are immersed under water for some time, development of the eggs proceeds at the advent of the rainy season, and infection of the intermediate host, if present, takes place. The sheep and cattle infect themselves by feeding on the grass or pasture along the margins of pools containing infected snails, and on which the cerceriae have encysted themselves.

In the south-western districts of the Cape Province infection of stock takes place during October, November, and December. In the eastern districts, viz. Molteno, Dordrecht, Barkley East, and wherever Paramphistomids (not necessarily *P. calicophorum*), occur in Natal, Orange Free State, and the Transvaal, infection takes place from April to June.

I had the opportunity of examining a large number of sheep infected with *Paramphistomum calicophorum* and *P. cotylophorum*. In one case I counted as many as 150 individuals in the rumen of an ox, and yet the host seemed none the worse for it, both as regards vitality and condition. Fortunately, therefore, no loss of stock infected with these parasites need be feared.

Paramphistomum calicophorum (Fisch.) (11) (fig. 9) is the species prevailing in the south-western districts of the Cape Province. I also have specimens from Dordrecht, Burghersdorp, and Molteno. It probably occurs all along the lower reaches of the Zumbergen and Drakensbergen into Natal.

Fischöder records it from Queensland, China, and the Cape Province (Kapland).

Paramphistomum cotylophorum (Fisch.) I have found in sheep from Dordrecht, Burghersdorp, and a few specimens were sent me from Onderstepoort.

The Life-history of Paramphistomum calicophorum Fisch.

(See figs. 1-8.)

The commonest fresh-water snail in the Cape Peninsula, and in the districts of Stellenbosch, Paarl, Tulbagh, and Ceres is the ubiquitous *Isidora* (*Physa*) *tropica* (Krauss). The eggs of the previous season, deposited

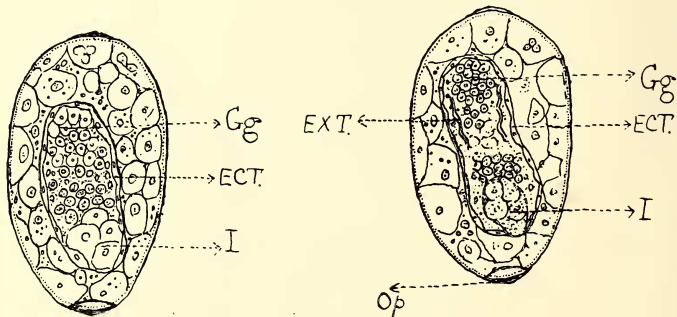


FIG. 1.

FIG. 2.

under the leaves of the common water-lilies (*Aponogeton*) and round the bases of stems of a dwarf variety of rush, hatch in July, in the middle of the rainy season.

During the months of August to November I undertook the examina-

tion of *Isidora tropica* in a restricted locality in what is locally known as the Stellenbosch Flats, situated in the town commonage. From previous observations carried out on the occurrence of rediae and cerceriae in fresh-

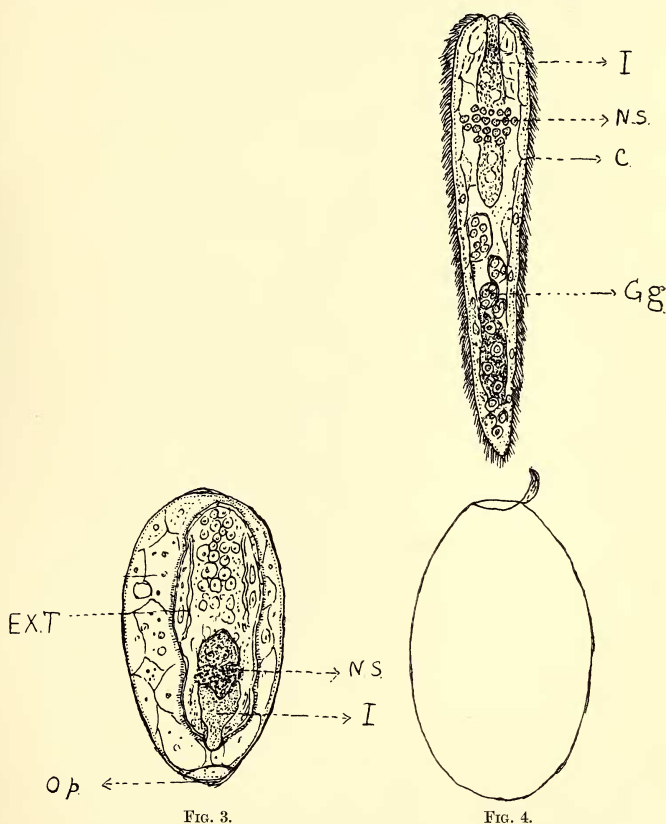


FIG. 3.

FIG. 4.

water snails from that particular locality, I knew that the snails harboured small black cerceriae in abundance. These cerceriae readily encyst on the grass stems growing round and in the pools of water, and thus easily reach the stomach of sheep and cattle that graze on the commonage. Animals that had grazed on the commonage were found to be heavily infected

with Paramphistomids about three months after they had grazed on the infected grass. It needed no particular ingenuity to regard the common *Isidora tropica* as the intermediate host of the locally known Paramphistomid. After a careful examination of the rediae and cerceriae in the

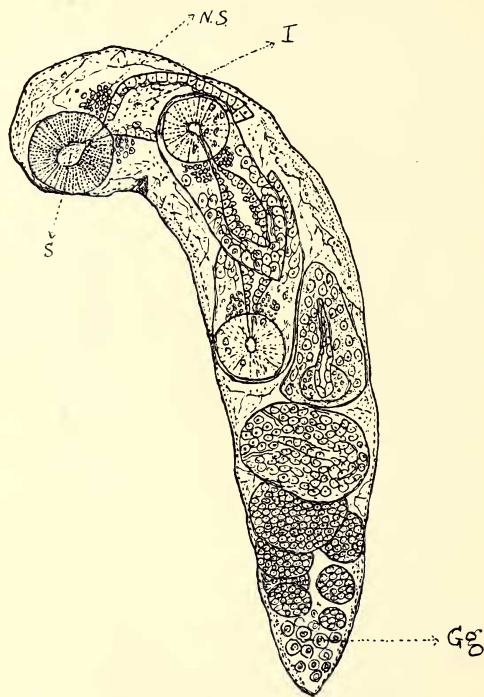


FIG. 5.

intermediate host *Isidora tropica*, I carried out a feeding experiment. Three young sheep and a young lamb were used for the purpose. The sheep were obtained from a dry locality in the Karoo from a small flock I knew to be free of Paramphistomids; the lamb I had reared myself, to make sure that it was free of infection from any source whatsoever. For three weeks, at the rate of two to three feeds per week, they were fed on grass heavily infected with encysted cerceriae. As a result of continued and careful

observations, it was quite certain that the snails from which the cerceriae had escaped to encyst on the grass used in my feeding experiment harboured only these particular dark-coloured cerceriae. In the locality selected there were no other species of fresh-water snails to be found; animal life in the pools, as far as vertebrates are concerned, was confined to the development of tadpoles that died off as soon as the pools began to dry up. The cerceriae encyst very readily, and in the laboratory I caused a large number

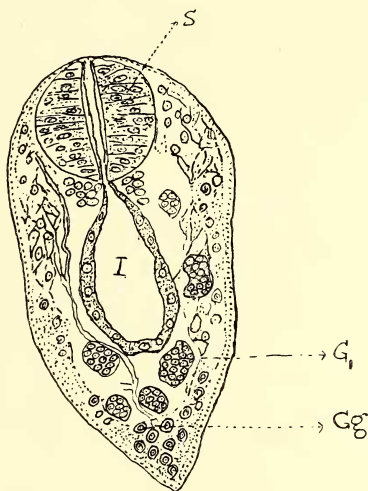


FIG. 6.

to encyst on grass stems placed in a wide tube of water into which the cerceriae of infected snails were removed. Such grass was also used as a feed. I was therefore perfectly sure that my experimental animals were fed only on the cysts of the cerceriae under observation.

A few months after the first feed I found on examination that my experimental animals were heavily infected with Paramphistomids. In some cases these flesh-coloured and cream-coloured parasites were found in all four divisions of the stomach, and a large number had found their way down to the small intestine. The death of one of the young sheep towards the end of the second month was evidently due to a confined life and an abnormal heavy infection which was revealed on a post-mortem examination.

The adult parasite I identified as *Paramphistomum calicophorum*

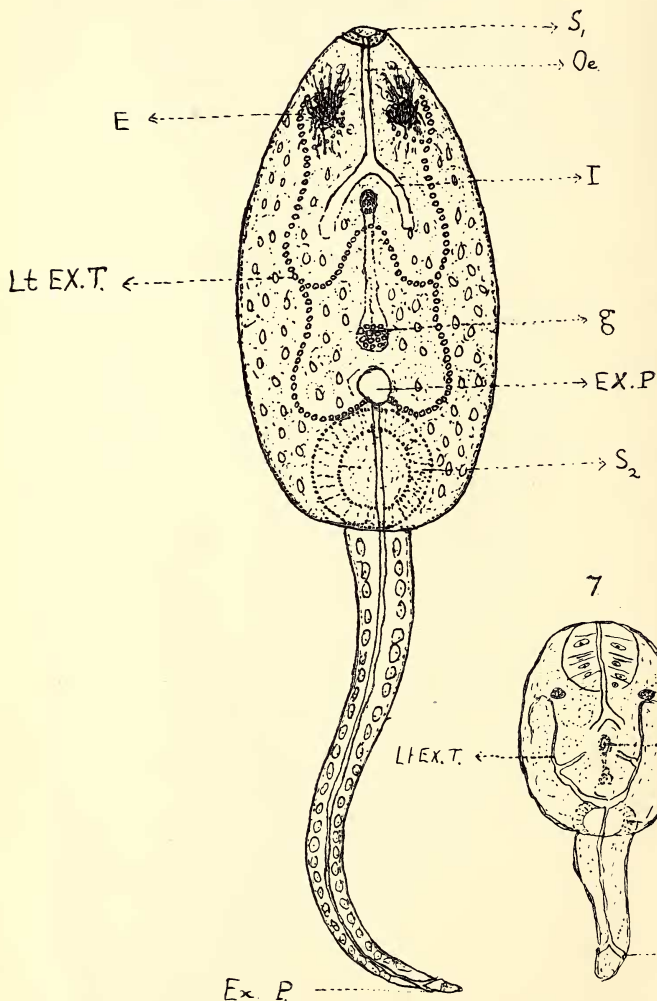


FIG. 8.

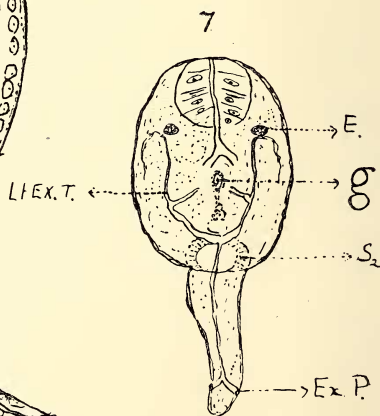


FIG. 7.

(Fisch.) (11), and the intermediate host is the small, dark-brown fresh-water snail *Isidora (Physa) tropica*, Krauss.

Observations on the Early Development of the Eggs of
P. calicophorum Fisch.

I collected a number of eggs in a flat dish, filled with water about half an inch deep. The eggs are light yellow, elliptical, tapering a little more

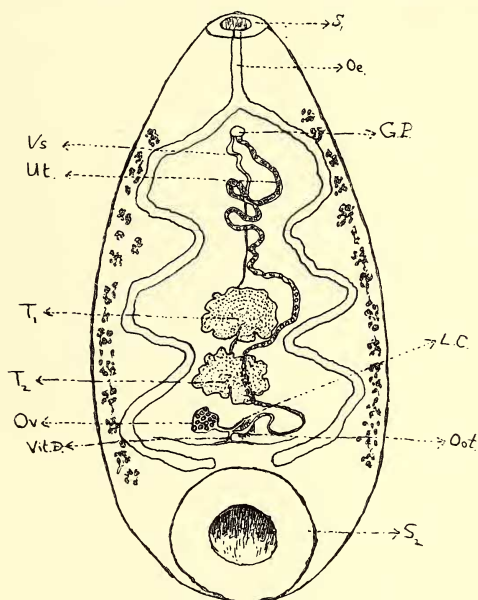


FIG. 9.

towards the opercular pole than to the opposite pole. Length .17 mm., greatest breadth .09 mm. The embryonic cells lie towards the opercular pole, and are surrounded on all sides by yolk-cells. The operculum is small. Development proceeded under water kept at room temperature (16°–17° C.). By segmentation the embryonic cells had on the eighth day extended to the opposite pole; the yolk-cells began to break up and diminish in size. A membrane of thin flattened cells had grown round the entire cell-mass, and one or two large covering cells lay over the anterior extremity of the

embryonic cell-mass. By the fourteenth day the embryo had become quite distinct: the segmentation cells had lost their outline, and the remains of yolk-cells were scattered round the embryo, which now almost filled half of the egg-case and extended from pole to pole.

On the twentieth day the embryo, the future miracidium, began to show slight movements of contraction and subsequent expansion. Evagination and invagination of the future alimentary canal were distinctly seen, accompanied by the ingestion of food-products into the embryonic digestive tract. The digestive tract could be clearly seen, on account of its lumen being filled with coarse granules.

On the twenty-third day the embryo had so much increased in size as to lie with its mouth right up against the operculum. The wriggling movements, the flickering vibration of the cilia, indicated that it had now merely to lift the operculum to emerge from the egg-case as a free-swimming miracidium. As soon as the eggs were now exposed to sunlight, the miracidium burst open the operculum and soon escaped to swim about in a smooth, swift-gliding fashion. It is, of course, well known, from experiments carried out by Leuckart, Thomas, Looss, and others, that sunlight is necessary for the miracidium to finally break through the operculum, and thence swim about freely in search of the intermediate host.

I now introduced a snail into the dish containing the miracidia. At first they swam about aimlessly, but as soon as they happened to come to about half an inch or so from the snail, they suddenly changed their course, swam straight up to the snail, and began to attach themselves to the exposed parts. Some soon let go their hold, swam away, again approached the snail, and attached themselves to it. Others, meantime, crawled over it with the definite object it appeared of entering somewhere. It was apparent that sooner or later some would find their way through the pulmonary aperture into the mantle cavity, encyst themselves round or in the kidney, or work their way up the visceral mass and encyst themselves in the liver.

As Looss (15) had as early as 1896 completely worked out the life-history of *Paramphistomum cervi* in Egypt, where *Physa alexandrina* (Bourg.) and *Physa micropleura* (Bourg.) are the intermediate hosts, I did not consider it worth while to actually infect snails for the purpose of my investigation. He found (*loc. cit.*, p. 186) that after a period of fifteen days the sac-like sporocysts contain immature rediae, and about fifteen days afterwards the first generation of rediae appear.

The Rediae and Cerceriae.

The rediae are colourless transparent organisms containing daughter-rediae in various stages of development. The more developed daughter-rediae are confined to the anterior region of the parent redia. The birth-

aperture is situated a short distance behind the sucker in the adult rediae. I counted as many as seventeen daughter-rediae in various stages of development in a parent redia. In many there are less. In some rediae one could determine immature from two to three cerceriae with their incipient but clearly defined eye-spots and well-defined tails, together with daughter-rediae; and, as one might expect, the immature cerceriae were confined to the anterior region and the daughter-rediae to the posterior extremity. It seems safe to conclude that the second generation of rediae give rise to rediae only, and the third generation give rise to rediae and cerceriae, and that the fourth and subsequent generations will behave as the third.

With regard to the development and generations of rediae and cerceriae, Looss (*loc. cit.*, p. 189) states in connection with the life-history of *Paramphistomum cervi* :—

“ Les produits de ces rédies commencent à se différencier de très bonne heure; ils prennent naissance et se développent de la même manière que les germes des rédies de l'*Amphistomum subclavatum*. . . . Mais, tandis que chez celui-ci les germes des premières rédies se transforment de suite en cercaires, chez l'*Amphistome conique* ($\times$ *Paramphistomum cervi*) la seconde génération donne encore des rédies. Ces dernières quittent leur mère par un orifice d'accouchement, situé à une courte distance en arrière de la ventouse; elles s'établissent à côté des rédies plus âgées, s'accroissent et produisent une troisième génération qui peut être encore une fois des rédies. De cette manière, il résulte finalement un assez grand nombre de rédies offrant à peu près le même aspect et qui commencent dès lors à produire des cercaires. Chez le ver qui nous occupe je n'ai pas des germes de cercaires en compagnie avec des germes de nouvelles rédies dans une rédie mère, comme cela se produit chez le *Gastrodisque*. Mais cela peut être purement accidentel, car je n'ai pu suivre, ainsi je l'ai dit, que pendant dense mois le cycle évolutif de ce ver.”

The mature rediae are .6 mm. to 1 mm. in length. The small anterior sucker is circular, with a diameter of .05–.07 mm. A daughter-redia just before birth is .25 mm. long, and the fairly well-developed sucker .05 mm. in diameter. The sac-like alimentary canal is well defined.

The cerceriae are born in a very immature state. They live for some time in the liver of the intermediate host, and when mature and capable of greater activity they leave the snail. In adult infected snails, as well as uninfected specimens, the shells are worn off at the apex, and a part of the liver is exposed. It is, therefore, certain that the adult cerceriae emerge from the intermediate host through the decaying apex of the shell. They may, of course, also leave the snail by the pulmonary aperture.

As soon as they escape they swim about actively in the water by the rapid wriggling movements of the tail. The body is almost entirely opaque

or black, owing to pronounced superficial pigmentation. This pigment is more pronounced in and round the eye-spots; it then extends forwards and backwards in heavy radiating streaks. In the young immature cerceriae the pigment is confined to the eye-spots a short distance behind the anterior sucker. The body of a mature cerceria is oval, .42 mm. long, and greatest breadth .25 mm. The tail is .5-.6 mm. long. The anterior sucker is small and circular, with a diameter of .03 mm., and the larger posterior ventral sucker has a diameter of .09 mm. The mouth leads into a slightly elongated œsophagus which bifurcates at a point just behind the eye-spots to form the two short limbs of the forked intestine. The tail springs from the dorsal posterior margin of the body just round the exterior margin of the posterior sucker.

The excretory system consists of two lateral trunks. Each main trunk starts anteriorly in the region of the eye-spots, runs downwards and outwards, and suddenly turns inwards. Here each trunk gives off a branch; the branches from each side meet to form a transverse trunk. The main trunks on each side now run outwards and backwards and join just in front of the posterior sucker. At the junction is a well-defined excretory pore, that opens on the dorsal surface. After the two trunks have joined they continue as a single tube down the tail bifurcating just before it reaches the distal extremity to open on each side through two minute pores.

Cystogenous cells are scattered throughout the organism. These cells aggregate to form chains around and along the course of the vascular trunks when the cerceria becomes less active and begins to encyst itself.

The cerceriae readily encyst themselves on the sides of the glass tube in which they may be collected, and on grass stems introduced into the tube. The tail is now cast off, the anterior and posterior extremities are drawn in under the body, and a small round black speck about .2 mm. in diameter is formed. Encystation is accompanied by the expulsion of a granular mass containing small dark rhabdite-like bodies. This granular mass hardens when exposed to the action of sun and air. Before encystation takes place the cerceriae crawl or wriggle up the grass stems for a short distance, and encyst just above the water-level.

I have no hesitation in regarding the cerceriae described above as identical with Cawston's *Cerceria frondosa* (7), which he removed from *Isidora schakoi* Jickeli, from Potchefstroom (Tol.). It is also the same as Gilchrist (13) recorded.

II. SOME TREMATODES IN SOUTH AFRICAN ANURA, AND THE RELATIONSHIPS AND DISTRIBUTION OF THEIR HOSTS.

It is a well-established fact that trematodes, as well as most parasitic worms, occur in fauna groups. If we investigate the trematodes in a particular class of host in a defined zoo-geographical region, we will find that closely allied or similar trematodes probably live in the same class of host in a different zoo-geographical region.

During the last four years I devoted a good deal of time to the examination of frogs in search of trematodes. In view of the cosmopolitan occurrence of the Anura throughout the Union, the present paper deals only with such trematodes as I have from time to time removed from frogs in the neighbourhood of the Cape Peninsula. A search for trematodes in frogs from the more inland districts, viz. Worcester, Middelburg (C.P.), Molteno, and Dordrecht thus far yielded no results. The material, however, which I have collected clearly bear out the truth of the above-mentioned statement.

Methods employed.—In looking for trematodes in frogs, I more or less followed the methods suggested by Johnston (14) and Looss (19). After inspecting the buccal cavity, the ventral body-wall was slit from vent to chin, the digestive tract laid open, and the whole length of the alimentary canal slit open, and the internal surface was examined with an eye-lens. The trematodes found in the body-cavity, gall-bladder, urinary bladder, lungs, etc., were immediately removed to glass dishes containing normal saline. Worms intended for whole mounts were placed in a drop of normal saline on a slide, and covered with a cover-glass or another slide according to the size of the worm. To prevent the cover-glass or the slide being washed away when the fixing fluid is added, it is necessary to put a small weight on the cover-glass or covering slide. The addition of a weight at the same time flattens out the object intended for a whole mount without appreciably changing its length. In the case of larger and stronger worms a good deal of pressure is often necessary to cause the worm to flatten out. In the case of small worms a small glass tube about 8 mm. in diameter and 20 mm. long was used; the pressure can then be regulated by the addition of a little mercury poured into the bottle. For larger and stronger worms small wooden cubes were placed on the upper slide and weights were then placed on these wooden blocks. In view of the action of fixatives on the metal weights, it is necessary to avoid such reactions by first using small wooden or glass cubes, and increase the pressure by adding small metal weights. The whole is now flooded with the fixing solution.

I obtained the best results by using boiling or a hot saturated solution of corrosive sublimate in water with about 5 c.c. of 2 per cent. glacial acetic acid to every 100 c.c. After a few seconds the weights are lifted off for a

moment, and one notices that the fluid has already taken effect by the opacity of the tissues round the edges. The weights are replaced, and finally removed after a period varying from five minutes to half an hour, according to the bulk of the worm.

The worms are further left in the fixing fluid for fifteen minutes and longer. They are subsequently washed in 40–50 per cent. alcohol, left for a day or so in iodised 70 per cent. alcohol, and they are then ready for the after treatment of staining and mounting.

I also obtained very good results by using 10 per cent. formalin as a fixative for whole mounts.

The following trematodes, referred to their systematic position, have been found :—

Order HETEROCOTYLEA Mont.

Family POLYSTOMIDAE Taschbg.

Sub-family POLYSTOMINAE Van Ben.

Genus *Polystomum* Zeder.

1. *Polystomum integerrimum* (Rud.) Fröl.?

In 1758 Roesel von Rosenhof (21) first figured and described this trematode from the urinary bladder of the frog without apparently naming it. In 1792 M. Braun (6) found it in the bladder of the green frog *Rana esculenta* (L.), and described it as *Planaria uniculata*, n. sp.

Zeder (25) in the year 1800 first attempted a classification of the then known trematodes. The various helminthologists had previously often described and figured the same animal under different names. The genus name *Planaria*, to cite one instance, was in turn used to denote entirely different worms. Zeder founded the genus *Polystoma*, and referred the above-mentioned *Polystomum integerrimum* to this genus as *Polystoma ranae*.

Rudolphi (22) was the first to describe and figure it under the name by which we now know it, viz. *Polystomum integerrimum* (he, however, wrote it *Polystoma integerrimum*).

Von Baer and Van Beneden (1827), Stieda and Willemoes-Suhm all subsequently contributed to its anatomy and development. It was, however, left to Zeller (26), who first in 1872 and afterwards in 1876 gave a complete description and fully worked out the life-history of this parasite.

Polystomum integerrimum is found in the urinary bladder of *Xenopus laevis* from Stellenbosch. About 5 per cent. of these frogs, popularly known as "platanas," harbour this parasite. I observed from one to six in the urinary bladder. Curiously enough, I never came across it in a large number of *Rana fuscigula* from Stellenbosch, Paarl, and Worcester.

Order MALACOTYLEA Mont.

Family FASCIOLIDAE Rail.

Sub-family $\begin{cases} \text{PLAGIORCHINAE L\"{u}he.} \\ = \text{LEPODERMATINAE Looss.} \end{cases}$

Genus *Opisthioglyphe* Looss (18).

2. *Opisthioglyphe endoloba* Dujardin.

Fr\"{o}lich (10) included this parasite with *Diplodiscus subclavatus* under the name *Fasciola ranae*. Rudolphi referred Fr\"{o}lich's *Fasciola ranae* to his own *Distomum clavigerum*, and in this was followed by a number of later writers, e.g. Dujardin and Diesing. Dujardin found the real *O. endoloba*, however, and, recognising it as distinct from *D. clavigerum*, described it in 1845 as *Distomum endolobum*. It was subsequently also described by Looss (17).

These small trematodes were found in the posterior region of the intestine, and in the rectum of *Xenopus laevis* from Stellenbosch. They are small oval worms with a body-length of 2-2.55 mm. Anteriorly the body tapers slightly more than posteriorly. The posterior extremity is slightly emarginate. I only came across it once. In about a half-inch length of the intestine, just before it opens into the rectum, I found as many as fifteen individuals.

O. endoloba is the common European frog parasite in *Rana temporaria*, occasionally in *Bufo variabilis*, *calamitas*, and *vulgaris*, and in *Triton cristatus*. The genus renamed by Looss (*loc. cit.*, Zool. Jahrb., p. 588) is closely related to the genera *Dolichosaccus* (Johnston) and *Brachysaccus* (Johnston) found in Australian frogs (14).

Sub-family SYNCOELINAE Looss.

Genus *Halipegus* Looss.

3. *Halipegus ovocaudatus* Vulpian.

Vulpian first records this trematode in 1860. It was subsequently described by Creutzburg (8) and Looss (18). Sonsino (1893) also found it in the stomach and in the anterior region of the intestine, whereas it is usually found in the buccal cavity under the tongue.

From the buccal cavity of *Rana fuscigula*, Stellenbosch.

Family PARAMPHISTOMIDAE Fisch.

Sub-family CLADORCHINAE Fisch.

Genus *Diplodiscus* Diesing.

4. *Diplodiscus subclavatus* Goeze.

Fr\"{o}lich (10) included *Opisthioglyphe endoloba* with *Diplodiscus subclavatus* under the name *Fasciola ranae*. Previously, in 1787, Goeze (12)

described it under the name *Planaria subclavata*. Rudolphi (22) placed it in his own genus *Amphistoma*. Diesing (9) established the genus *Diplodiscus*, and referred *Amphistoma subclavata* to *Diplodiscus*, calling it *Diplodiscus subclavatus*. Later authors—Filippi, 1855; La Valette de St. George, 1855; and Pagenstecher, 1857—devoted themselves to observations on its anatomy and life-history. Von Linstow described the reproductive system fully, and observed the independent movements of the vitellogenous granules. It was Looss (16), however, who, in 1892, published a complete account of this well-known parasite and fully established its life-history.

Judging from the size of the suckers, length of intestinal limbs and shape of the testes, I found the South African specimens from the rectum of *Xenopus laevis* (Stellenbosch, Paarl, and Kuilsrivier) identical with the European representatives. The Australian representatives constitute the species *D. megalochrus* Johnston and *D. microchrus* Johnston (14).

I wish here to record the discovery of two other trematodes found in *Xenopus laevis*, which I have thus far been unable to refer to their natural position among the Malacotylea. The following is a preliminary account.

*Preliminary Account of a Distomid found in the Gall-bladder
of Xenopus laevis.*

The presence of these worms in the gall-bladder of *Xenopus laevis* imparts a pronounced yellowish-green colour to that organ. On opening up the gall-bladder the worms were found shrouded in a dense mass of a partly coagulated substance. They occur singly or in pairs, more often in pairs. The body, about 7 mm. long, is divided into two distinct regions of more or less equal length: an anterior narrow region .5 mm. wide, and a posterior, flattened, oval region about 2 mm. wide. Posteriorly it ends in a moderately smooth curve. The anterior half is mobile, carrying the anterior and posterior suckers, of which the former has a diameter of .17 mm. and the latter of .1 mm.

It has a well-developed bulbous pharynx, short œsophagus, and intestinal limbs which do not extend to the posterior extremity. The testes are rounded, and placed symmetrically at the same level on each side of the body just within the intestinal limbs in the posterior region of the body. The compact rounded ovary is almost symmetrically placed in front of the testes, slightly to the left of the middle line. The coils of the uterus, filled with yellowish-brown eggs, are confined to the region between and behind the testes, and do not extend over the intestinal limbs on each side. The vitellaria are lateral, extending from the level of the posterior sucker to a point a short distance in front of the termination of the intestinal limbs. They form dendritic clusters arranged on each side external to the intestinal

limbs, and only overlapping the limbs anteriorly. A vesicula seminalis is present. The genital aperture is situated between the suckers, nearer to the posterior sucker.

With respect to such external characters as the division of the body into a narrow mobile anterior and a broad flattened posterior region, it certainly belongs to the Gorgoderinae (cf. *Spathidium folium* v. Olfers), but it differs from them in the presence of a well-developed pharynx, short oesophagus, vitellaria confined to the sides, and a poorly developed posterior sucker.

It differs from the Brachycoelinae in respect to pharynx, length of intestinal limbs, and position of the vitellaria, but agrees with them in respect to the position of ovary and testes and the coils of the uterus confined to the region behind the testes. It certainly shows affinities with both the Gorgoderinae and Brachycoelinae. I am of opinion that it is more closely allied to the latter than to the former.

*A Distomid from the Body-cavity of Xenopus laevis belonging to
the genus Heterolope Looss (sp. inq.).*

This trematode I found in the body-cavity of *Xenopus laevis*. On one occasion I found it under the peritoneum on the outer surface of the lungs. The worm measures 9 mm. from end to end, greatest breadth 2 mm. It tapers more posteriorly than anteriorly. The suckers are well developed, and approach each other. The ovary lies between the testes, which are situated one behind the other in the middle line in the posterior region of the body. Cirrus-sac, ductus ejaculatorius, and cirrus present; a vesicula seminalis lies outside the cirrus-sac. The genital aperture lies just in front of the anterior testes in the posterior region of the body, far behind the posterior sucker. The coils of the uterus lie in front of the testes.

So far I have not been able to find a frog-trematode recorded in which the genital aperture lies far behind the posterior sucker in the posterior region of the body. It undoubtedly belongs to the genus *Heterolope* Looss, or a closely allied genus.

General Conclusions.—In considering the relationships and distribution of the hosts of South African frog-trematodes, one can, it appears to me, only arrive at such conclusions as Johnston (14) did with respect to the Australian frog-trematodes. The following is a reproduction of his conclusions so modified as to include the South African representatives.

The occurrence of the trematodes recorded above from South African frogs gives a striking illustration of the tendency of helminths to occur in faunal groups. That is to say, that helminths found parasitic in any

particular class of host, in a defined zoo-geographical region, find their nearest relatives not in that region in which they themselves occur, but in the same class of host living in other zoo-geographical regions.

Five such faunal groups from Amphibia are now more or less well known, viz. European, North American, Asiatic, Australian, and South African. In each region we find, in hosts of this class, trematodes corresponding or closely corresponding with representatives in the other regions. The group in the European region, as the longest known and most extensively investigated, shows the greatest number of genera; in point of numbers, the American group, to which a good deal of attention has been given of late years by Stafford and others, follows closely on the European. The Australian, Asiatic, and South African groups show smaller numbers, partly, perhaps, because less completely worked up. The facts at present seem to indicate that in Asia, Australia, and South Africa the number of representatives may be further, more or less extensively, added to by subsequent investigations. Besides the frogs, other classes of hosts, as mammals, birds, and reptiles, show faunal groups of parasites with relations analogous to those exhibited by the group from frogs, as the small collection of trematodes I have from mammals thus far go to show, viz. *Paramphistomidae* and *Fasciolidae* from sheep and cattle, for example. Leaving the latter in the meantime out of account, and confining ourselves to the groups from frogs, we find: *Opisthioglyphe endoloba*, occurring in the intestine of European and South African frogs, is represented in North America by *Glypthelmius quieta* and in Australia by *Dolichosaccus trypherus* and *Dolichosaccus ischyryus*, which also live in the proximal part of the intestine of their hosts. The three European species of *Pneumonoeces*, found in the lungs, are represented in America by no less than six species (*P. longiplexus* Stafford, *breviplexus* Stafford, *P. varioplexus* Stafford, *P. similiplexus* Stafford, *P. medioplexus* Stafford, and *P. complexus* Seeley), while they are represented in Asia by *Pneumonoeces capyristes* Klein, in Australia by *P. australis*. The only representative of *Pneumonoeces* I have thus far come across was a specimen given me by my chief, Dr. E. J. Goddard; it was removed from the lungs of *Rana fuscigula*. Unfortunately it was damaged, and did not lend itself to specific determination.

The *Gorgoderinae*, represented in European frogs by the two genera *Gorgodera* and *Gorgoderina*, comprising between them, according to Ssinitzin, five separate species, all living in the bladder of frogs, are represented in America by four species of *Gorgoderina* and one of *Gorgodera*. No species of *Gorgodera* has yet been described from Asia, and thus far I have not yet found any in South Africa, but the genus is represented by one species—*G. australiensis* (Johnston)—from Australia. The European *Brachycoelium crassicolle* R., found in the intestine, is represented in America by *B. hospi-*

tale; in Australia by three species of *Mesocoelium*, viz. *Mesocoelium mesembrinum* (Johnston), *Mesocoelium megaloon* (Johnston), and *Mesocoelium oligoon* (Johnston); in Asia by *M. sociale*; and the form I have preliminarily described from the gall-bladder of *Xenopus laevis* may turn out to be the South African representative of the *Brachycoelinae*.

The *Pleurogenetinae*, occurring in the anterior part of the intestine, are represented in Europe by three genera (*Prosotocus*, *Pleurogenes*, and *Brandesia*), comprising between them seven species; in America by *Loxogenes arcanum*, in Asia by *Pleurogenes gastroporus* and *P. sphaericus*, and in Australia by *P. freycineti* and *P. solus*. *Halipegus ovocaudatus*, occurring in the buccal cavity of European frogs, is also represented in South Africa; in North America represented by *H. occidentalis*; in South America by *H. dubius*; and in Asia by *H. longispina*—all living in similar situations; but, up to the present, no representatives of this fluke have been found in Australia.

Diplodiscus subclavatus in the rectum of European and South African frogs is represented in America by *D. temperatus*, and in Australia by two species of *Diplodiscus*. The European *Polystomum integerrimum* in the bladder of frogs does not seem to be represented in America in frogs, but three species (*P. coronatum* Leidy, *P. hassalli* Goto, and *P. oblongum* R. Wright) occur there in the bladders of Chelonians; in Australia this heterocotylean genus is represented by *Polystomum bulliensi* Johnston, and in South Africa by the European species. No representatives of *Diplodiscus* nor *Polystomum* have yet been described from Asia. The American *Cephalogonimus americanus* in the intestine of frogs may be represented in the Old World by *C. lenoiri* Poir. The genus *Ganeo*, described by Klein from the Indian *Rana hexadactyla*, seems to stand alone, unrepresented in any other region. Likewise the "species inquirenda," belonging to the genus *Heterolope*, from the body-cavity of *Xenopus laevis*, referred to above, seems to be unrepresented in the frogs of other zoo-geographical regions.

It is a remarkable fact that, of the six species of flukes described from frogs inhabiting Southern Asia, four of them appear to find their nearest relatives in flukes from Australian frogs. *Mesocoelium sociale* Lühe is certainly more closely related to the Australian species of *Mesocoelium* than to *Brachycoelium crassicolle* R., its European, or *B. hospitale* Stafford, its American representative. *Pneumonoeces caprystis* Klein has been shown (Johnston, *loc. cit.*, p. 325) to be more nearly related to *P. australis* than to any other European or American species of this genus, and the Asiatic *Pleurogenes gastroporus* and *P. sphaericus* and the Australian *P. freycineti* and *P. solus* have likewise been shown to be more nearly related to one another than any of them are to the American or European *Pleurogenetines*. The South African group, on the other hand, is certainly directly related to

the European frog-flukes, the Asiatic, as it were, standing midway between the Australian and European. The American frog-flukes, many of which have evolved into distinct genera, are not so nearly related in their structure to the European as are the Asiatic. And in addition to this, the American genera, generally speaking, contain more species than the same genera in Asia, Australia, and South Africa, and this may be taken to indicate that the American frogs, with their flukes, have been longer separated from the parent-stock.

The great similarity of the five groups of flukes from frogs found in the five regions mentioned, points to the fact that the flukes are a very old group of animals, and existed in the ancestors of present-day frogs a very long time ago, when their distribution was much less extensive than it is to-day. The mutual relationships of these groups of trematodes support the view that the *Anura* originated somewhere about the centre of the Palæarctic region, and migrated westwards, southwards, and south-westwards. They may have reached the western portion of the Boreal land-mass, existing right across from Asia to North America, in early Tertiary times; or they may have made their way westwards in Pliocene times, when a considerable migration of vertebrates westwards is known to have taken place. The Australian forms must have found their way there before the separation of the Australian continent from South-eastern Asia, a separation which is generally supposed to have taken place somewhere about late Cretaceous or Eocene times. The South African forms must have found their way down here during late Pliocene times. The greater diversity of the North American frog-trematodes would seem to indicate that they have been longer separated from the parent-stock than the Asiatic, Australian, and South African forms, so that the America-wards migration probably took place in the earlier of the two periods mentioned.

In view of the probable land connection between Australia and South America through the Antarctic, a connection which is supported by a good deal of biological evidence, it is unfortunate that practically nothing seems to be known about the frog-trematodes of South America. There are only two indirect references to such trematodes, viz. in Braun (2, p. 906) and Klein. A pretty close similarity has, however, been shown by Zschokke to exist between some cestode-parasites of South America and the Australian Marsupials.

The close similarity existing between the respective representatives in the five groups of frog-trematodes in question here reminds us that the trematodes, owing to the conditions under which their lives are passed, have probably evolved much more slowly than their hosts, for the Amphibian ancestors of our own present-day groups, at the time of

their dispersal, must have been considerably different from their representatives now living.

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EXPLANATION OF FIGURES.

Figs. 1-4 illustrate the development of the egg up to the hatching of the free-swimming miracidium emerging from the egg-shell.

I. = Intestine.

E.C.T. = External cellular envelope.

Gg. = Germ-cells and yolk-cells.

Ex. T. = Excretory tube.

Op. = Operculum.

N.S. = Cells of nervous system.

C. = Ciliated coat.

S. = Sucker.

G.₁ = Developing mass of germ-cells to give rise to redia.

Fig. 5. A redia of the first generation.

Fig. 6. An immature redia of the second generation.

Fig. 7. An immature cerceria just after it has escaped from the redia.

Fig. 8. A mature free-swimming cerceria, *Cerceria frondosa*.

Fig. 9. Mature *Paramphistomum calicophorum* Fisch.

S.₁ = anterior sucker.

S.₂ = posterior sucker.

Oe.₂ = oesophagus.

Vs. = Terminal region of vas deferens

Ut. = uterus.

T.₁, T.₂ = Testes.

Ov. = Ovary.

Vit. D. = Right vitelline duct from lateral vitelline gland.

Oot. = Ootype.

L.C. = Laurer's canal.

G.P. = Genital aperture.

The figure represents a ventral view with posterior extremity slightly turned up to show posterior sucker.